CENTROSOMES IN PLANTS.

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THERE are few questions in cytology to which so much attention has been paid in recent years, especially by zoologists, as that of centrosomes and directive spheres. First observed, as is known, in animal cells undergoing division, they were later also found in the same cells in a resting stage. The presence of centrosomes in plants had not been demonstrated before my investigation of the subject. But, according to recent work, instead of being of general occurrence in plants, as was believed, they may be limited to the lower groups of the plant kingdom. However, in a subject so difficult, in which the record of new observations may be expected daily in consequence of the incessant progress of investigation, I do not believe absolute assertion of such an opinion is prudent.

Without entering into details, it will suffice to recall first that in animal cells the morphological and microchemical characteristics of the attractive spheres and centrosomes may be far from uniform. It is at least agreed that the centrosome is to be considered the important part of the sphere. Usually it is a refractive corpuscle, sometimes of extreme smallness. The sphere itself is formed of a substance which can be differentiated into two zones; one clear toward the center, the other granular toward the periphery. Also, it is often poorly defined, and of a refrangibility different from that of the surrounding protoplasm. The radial striation which usually surrounds it has its origin partly in the centrosomes, partly in the central region of the sphere.

In animal cells in the resting stage the sphere is often lacking, or is indicated only by a slight protoplasmic condensation around the centrosome. Sometimes it is even disorganized and

then reorganized before the division of the nucleus. It is formed of that substance which certain authors have called archoplasm, or kinoplasm, in order to distinguish it from the nutritive protoplasm or trophoplasm. It can be said in a general way, therefore, that the centrosome is the fundamental element of the sphere, since often it alone persists, and it is the centrosome which has the greatest affinity for staining fluids. However, it is known that in certain cases, observed especially by Van der Stricht and von Rath, the attractive sphere conceals the centrosome, but one is not justified perhaps in concluding that the latter element is in such a case completely absent.

Some observers have asserted, also, that the centrosomes and the spheres have no distinct existence, but are appearances resulting from certain activities of the protoplasm.

It does not seem possible at present to characterize the centrosomes by their composition, or by the manner in which they behave in respect to stains. Yet Heidenhain, and other authors claim that the centrosomes are composed of a particular chemical substance; while Viessing thinks that to find the specific character of these bodies it is necessary to study, not their chemical composition, but their position in the center of a protoplasmic radiation which they serve as a point of attachment.

In the resting animal cell there has been observed a single centrosome, sometimes two neighboring centrosomes, sometimes even numerous centrosomes lying side by side and forming the "microcentre" of Heidenhain, or the "centriole" of Boveri. In this last case the typical centrosome is replaced by a group of granules, which divides, like a typical centrosome, into two at the beginning of nuclear division.

Often the centrosomes, very small and scarcely visible at the moment of nuclear division, increase in size during that process, divide at the same time as the nucleus, and then return to their former condition and cease to be recognizable. There are several authors, however, who do not consider the centrosomes permanent organs of the cell, but such is not the opinion of the majority of zoologists, who have observed centrosomes

in cells of very diverse nature and in the period of complete rest. Their appearance in animals seems general and in all phases of the life of the cell.

It may prove, however, not to be the same with plants. The observations of Farmer, of Strasburger and his pupils, tend to show that among plants these elements occur only in the thallophytes and lower bryophytes ; and that they may not exist at all in vascular cryptogams and phanerogams. Such a difference between thallophytes and cormophytes on the one hand, and between the latter and animals on the other, is somewhat surprising when one considers the analogies presented in the fundamental structure of the cell, especially in the phenomena of nuclear division, in the great majority of plants and animals. However, the question may depend wholly upon the difference between purely theoretical views.

Among lower plants in which centrosomes have been described by the preceding observers, they present morphological variations analogous to those with which we are familiar in animals. For example, in Fucus, in the developing oogonia and the segmenting eggs, Strasburger has observed centrosomes surrounded by well differentiated radial striations. Swingle has made similar observations in the vegetative cells of Sphacelaria, with this difference, that instead of having the form of a rounded granule, the centrosome resembles a little box (boitonnet), a dumb-bell, or is club-shaped. This centrosome divides, persists during the resting period, and varies but slightly in size. Among these plants the centrosome seems to be without any clearly differentiated surrounding sphere, but the sphere has been observed by Farmer and Strasburger in some of the Musci.

Among fungi (Peziza, Ascobolus, Erysiphe) Harper has found, instead of an ordinary centrosome, a granular body of discoid form from which extend radial striations. According to Fairchild Basidiobolus has barrel-shaped spindles; that is, truncated at their polar extremities, and composed of several strands of threads, each strand being terminated by a distinct granule which stains readily. Similar strands have been observed in

animals (Ascaris, Cyclops, etc.), but here it was a form merely transitory which precedes the normal bipolar stage which, when attained, shows centrosomes of the usual type.

These observations all go to prove that our conception of centrosomes should be much broader and more general than at the beginning of our acquaintance with them.

One of the principal arguments against the existence of centrosomes among the cormophytes is drawn from the method of formation of the nuclear spindle. Many observers have stated that the spindle, instead of being bipolar from the first, begins with a variable number of poles; often more than a dozen, according to Osterhout, in the spore-mother cells of Equisetum; a half dozen or less, according to Mottier, in the pollen mother cells of Lilium, Podophyllum, etc. But at a fixed time these multipolar spindles always become bipolar, either by fusion, or by the drawing in of the filaments which form the multiple cones of the primitive figure. Neither at the summit of these multiple cones, nor at the extremities of the bipolar spindle derived from them, have these authors seen anything showing the character of a centrosome. It may be asked, then, what are the forces which thus change the multipolar to the bipolar spindles. If, according to the preceding observers, neither centrosomes nor anything capable of their function is present, one must invoke a mechanical explanation for these changes. Can it be the chromosomes which orient the achromatic threads of the spindle? Well-known facts tend to show precisely the contrary. If one believes in the absence of any kinetic center, it only remains to admit with Strasburger that the forces in play reside in the kinoplasm, independent of any special morphological differentiation. The opinion of a distinguished scientist may perhaps be given some weight.

Even though all earlier observations upon the presence of attractive spheres and centrosomes in different cormophytes may be regarded as inexact, one cannot doubt that the bodies recently described and figured by Webber in the pollen cells of Zamia, although they serve at a given moment in the formation

of the cilia of the antherozoids of this cycad, are centrosomes; and the same thing appears likely to be the case in Gingko, studied earlier by Hirase.

The following are the results of my recent investigations of the pollen mother cells of various phanerogams (*Nymphæa alba*, *Nuphar luteum*, *Limodorum abortivum*), examined after fixation in Flemming's fluid and other suitable reagents.

In Nymphæa, which is of particular interest, the adult mother cell contains an excentric nucleus, lying very near the wall, the rest of the cell being almost entirely filled with starch. At the commencement of the prophases of division the cytoplasm assumes a filamentous appearance around the nucleus, in which the chromosomes (apparently thirty-two in number) are peripherally placed in contact with the nuclear membrane, and in addition to the nucleolus a certain number of very fine achromatic threads can be distinguished.

In the neighborhood of the nucleus certain stains, such as a mixture of methyl-green, fuchsin, and orange G, bring out one or two small differentiated bodies, sometimes of homogeneous aspect, sometimes provided with a more deeply stained central granule and sometimes more numerous. At certain stages they act as points of attachment for cytoplasmic threads directed towards the nucleus, and form the first outline of the nuclear spindle. Sometimes tripolar or even quadripolar spindles are found, but the final figure never has more than two poles, occupied by a small sphere which has for its center either a single granule or several granules in a group, whose deep green color, brought out by the stain indicated, is like that of the chromosomes. These elements can also be stained by other methods. On account of the small number of multipolar spindles that I have observed, I am not able to suggest, at present, in what manner the multipolar spindles become bipolar.

The nuclear spindle forms on the side of the cell, becoming curved in accordance with its contour. The two cones which compose it, and whose bases correspond to the nuclear plate, elongate and incurve more and more, until the spindle becomes

crescent-shaped, and sometimes twisted upon itself in the form of an S. The length of the crescent often equals half the circumference of the cell. This singular elongation appears to depend upon polar bodies which always occur at the points of the spindle. During the process sometimes these bodies are represented by a sphere with one or more granules, and sometimes the sphere does not appear stained as the rest, a difference which may be attributed, in part, to the variable action of the reagents. In every case these bodies certainly represent centrosomes. Striations are also often to be seen extending from them into the cytoplasm.

Often, also, the ends of the spindle are extended into a slender point, formed by several granules disposed in a line. One might think that in this case there is no centrosome at the pole, but, in my judgment, it is to be explained by the dissociation of the primitive centrosomes, whose numerous granules have disposed themselves in this way. The reaction of these elements to stain certainly suggests this view.

The centrosomes are found in all the phases of nuclear division. After the formation of the daughter nuclei they can still sometimes be seen, becoming indistinct but not actually disappearing. They are found during the second division of the mother cell.

In Nuphar the nucleus is not lateral, but occupies a central position in the cell, which is completely filled with starch grains. The cytoplasm presents an alveolar appearance. The character of the nuclear division differs from that in Nymphæa in being of the normal type. It is to be remarked that the chromosomes are twelve in number, their arrangement in the nuclear plate stage being in groups of four, observed by Calkins in the ferns, and by many zoologists in the copepods and other animals. At the beginning of the prophases multipolar spindles are also found, at whose extremities there is very often a granule or a more or less distinct sphere serving as the point of departure for achromatic threads. At later stages the polar bodies or centrosomes resemble those of Nymphæa, but the bipolar spin-

dle, formed at the center of the cell, always lies as in ordinary cases.

It is exceedingly difficult to detect the bodies representing centrosomes in Limodorum. Multipolar spindles are very frequent, and in general their branches are very numerous and very variable in appearance. At the extremity of many of them, however, there is seen a granule, or a small mass of substance more stained than the rest of the cytoplasm. During the formation of the bipolar spindle, those granules nearest the points subsequently occupied by the two final poles, points determined by the form of the cell, seem to persist, the others disappearing. It can be supposed that several isolated bodies sometimes unite to form a single centrosome, for, after the formation of the bipolar spindle, bodies are often seen at each pole. A distinct sphere was not observed, but time after time very delicate radiations from each pole were seen.

To summarize, I may say that it does not appear permissible to advance the formation of multipolar spindles, which may be accidental or normal, as an argument against the existence of dynamic centers during nuclear division. It remains a fact that at a certain time there appear in the cytoplasm bodies distinct from the ordinary granules. It is possible that the construction of the multipolar figures may be independent of the elements which form centrosomes. It may also be true that centrosomes are not always definite morphological units. But it is none the less certain that the higher plants possess differentiated elements whose rôle is the same as that of those analogous bodies observed in the lower plants and in animals.

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